CARPMAELS & RANSFORD

CHARTERED PATENT ATTORNEYS EUROPEAN PATENT ATTORNEYS TRADE MARK ATTORNEYS

TELEPHONE 020-7242 8692
FACSIMILE 020-7405 4166
WEBSITE www.carpmaels.com
E-MAIL email@carpmaels.com

43-45 BLOOMSBURY SQUARE
LONDON WC1A 2RA

AND AT MUNICH

European Patent Office
Erhardtstrasse 27
D-80469 Munich
GERMANY

YOUR REF

OUR REF P036148WO:HRG

12th October 2005

Dear Sirs,

Re: International Application No. PCT/IB2004/004335
University of Groningen et al.

I refer to the Demand under Chapter II PCT that was filed in connection with the above-referenced patent application. In response to the Written Opinion of the International Searching Authority, I hereby file the Applicants' written reply to the Opinion. We appreciate that these arguments have been filed outside the official time limit for providing comments to the EPO, but trust that in view of the short time period that has elapsed since the filing of the Demand under Chapter II PCT, the EPO will not yet have begun to draw up the International Preliminary Examination Report. Assuming that this is the case, pursuant to Rule 66.4bis, we understand that arguments in response to the opinion expressed in the WO/ISA may still be take into account and we respectfully request that this be done.

An amended claim set is provided herewith to replace that previously filed under Article 19 PCT and we request that International Preliminary Examination be based upon these claims. The claims are now limited to mutants of the TRAIL cytokine. Furthermore, the limitations of previous claim 5 have been introduced into claim 1. Claims 17 and 19 (previous claims 20 and 22) now recite particular mutations that cause alteration of the selectivity of the TRAIL protein for its receptors. Claim 29 (previous claim 35) incorporates the limitations of new claims 1 and 17.

The Examiner is of the opinion that the principal claims of this application as initially filed relate to cytokine mutants that are defined only by reference to a result to be achieved.

FACSIMILE MESSAGE

To: **EPO Munich**
Fax No.: **00 49 89 2399 4465**

This fax consists of 11 sheets. If a sheet is missing or has been imperfectly received, please contact us immediately (Tel: 020-7242 8692; Fax: 020-7405 4166). If you are not the addressee, please contact us immediately and then destroy this fax.

We respectfully submit that this is not the case, particularly taking into account the amendments now made to the claims.

Amended claim 1, for instance, defines an improved β sheet multimeric cytokine with enhanced stability over the wild type cytokine. This enhanced stability is achieved by mutating a residue in a monomer component of the multimeric cytokine protein so as to improve the free energy of the monomer or of the multimeric complex relative to the wild-type unmutated monomer component by mutating either a) a non-conserved residue at the surface of the monomer component of the multimeric cytokine; b) a non-conserved residue close to the interface between two of the monomer components of the multimeric cytokine; c) a non-conserved residue along the central trimeric axis; or d) a miscellaneous residue whose mutation is energetically favourable. These are the technical features of the mutants that lead to the described technical effect i.e. improved stability. The claim does not therefore merely specify that the claimed cytokines have improved stability – it specifies how this improved stability is achieved. A *caveat* to the claim specifies that the mutated residue is non-conserved between homologous members of the cytokine family as, in contrast to similar studies in the prior art, the inventors found that it was important not to mutate conserved residues as this disrupted the biological function of the cytokine.

Similarly to claim 1, claims 17, 19, and 29 are directed to improved β sheet multimeric cytokines that show selectivity for target receptors and specify how this result is to be achieved. For example, claim 17 specifies that one or more amino acids that are located in the receptor-binding interface of the cytokine are substituted for replacement residues that include amino acid side-chain conformations that are predicted, according to the invention, to fit into the binding interface with the target receptor so as to provide an increase in binding affinity and selectivity/specificity of the cytokine protein for that target receptor and specific mutations are listed. Again, a *caveat* to the claim specifies that the mutated residue is non-conserved between homologous members of the cytokine family. Similar technical features are evident in the other independent claims.

We understand the Examiner's position that an effect on stability or receptor selectivity need not actually be documented for novelty to be destroyed as such an effect may be inherent. However, for novelty to be destroyed, the described effect must exist, albeit inherently, otherwise any mutant cytokine would destroy the novelty of the claims and this cannot be the case.

Taking the specific case of claim 1, for novelty to be destroyed, the prior art must describe a cytokine that is more stable than the wild type cytokine as a result of the technical feature specified in the claim i.e. the existence in the protein sequence of a residue in a monomer component of the multimeric cytokine protein mutated so as to improve the free energy of the monomer or of the multimeric complex relative to the wild-type unmutated monomer component.

The prior art is silent in terms of any of these effects.

D1:WO 03/029420 A (GENENTECH, INC)

This patent application describes TRAIL variants comprising one or more amino-acid substitutions for the specific purpose of chemical modification (e.g. "pegylation" with poly ethylene glycol (PEG)) of these variants. Amino acid substitutions in described TRAIL

variants comprise of 18 surface exposed amino acids towards cysteine (Cys) or, in one specific case (position 170), towards cysteine (Cys), serine (Ser) or Lysine (Lys). These specific amino-acid substitutions were chosen in order to provide reactive groups for chemical modification. No cytokine is described that satisfies any of the requirements of the claims.

D2: WO 99/36535 A (GENENTECH, INC)

This patent application describes a soluble part (amino acids 91-281) of the TRAIL native sequence, chimeric and pegylated variants hereof and various host cells for production purposes. Furthermore, it describes three aspartic acid to alanine substitutions (positions 203, 218, 269) and one valine to alanine substitution (position 207); these variants show effects on biological activity when compared to native TRAIL but variants did not show any significant change in stability or binding characteristics towards the TRAIL receptors DR4, DR5 or DcR1 when compared to native TRAIL. Accordingly, this disclosure do not provide any teaching with regard to modifying receptor selectivity or improving (thermodynamic) stability. The only improved binding that is shown is tighter binding towards the OPG receptor; this is in fact an unfavourable property.

D3: WO 01/00832 A (GENENTECH, INC)

This patent application describes formulations of native TRAIL and one or more divalent metal ions. Production and purification methods are also described. In addition, several alanine substitution variants of TRAIL are described, although mutations were restricted to alanine, using the technique of alanine scanning. No rational mutagenesis strategy was performed, in stark contrast to the techniques used in the present invention.

Certain effects on biological activity and binding towards TRAIL receptors DR4, DR5, DcR1 and OPG are described. Alanine substitutions at position 193, 195, 259, 264 and 267 resulted in a more than five-fold decreased affinity for DR4 but a small effect on DR5 binding. However, it was concluded that most alanine substitutions had similar effects on DR4 and DR5 binding and none of the alanine variants showed improved binding to DR5.

Other positions were considered not to influence receptor binding (or selectivity) significantly. In summary, this would have discouraged an investigator to pursue these positions in order to obtain TRAIL variants with improved DR5 binding or improved receptor selective binding characteristics, respectively.

D4: WO 88/06625 A (CETUS CORPORATION)

This patent application describes arginine depleted variants (either by deletion or substitution) of tumour necrosis factor for the specific purpose of improving the handling properties or decreasing susceptibility towards proteolytic enzymes. Only positions 2, 6, 31 and 32 are mentioned for this purpose. In addition, substitution of cysteine residues at position 69 and 101 are mentioned for the purpose of decreasing susceptibility towards oxidation. In any event, due to sequence divergence between tumour necrosis factor and TRAIL, there is no extrapolation to TRAIL possible.

D5: W0 2004/001009 A (GENENTECH, INC)

This patent application is potentially citable against the novelty of the claims of the present application, although this cannot be assessed at the present time. We note, however, that this patent application describes TRAIL variants obtained by phage-display. Information from the alanine scans in D2 and D3 is used to construct mutagenesis libraries. Isolated receptor selective variants have multiple (~6) amino acid substitutions. The results described in document D5 are substantially different from those illustrated in the present application, both in the number of substitutions that are required and the specific substitutions - the isolated variants in D5 require multiple amino acid substitutions and at positions that are different from those presently claimed. Different mutations are described, in case of substitutions at the same amino acid positions.

CONCLUSION

The cited documents do not provide any teaching to direct the skilled person to obtain TRAIL variants with improved (thermal) stability or TRAIL variants with altered receptor binding characteristics (more DR4 or DR5 selective).

In particular, the alanine scan data of TRAIL described in D2 and D3 did not reveal anything in relation to (thermal) stability and/or receptor selectivity/specificity. In many cases, the substitution to alanine was not physiologically relevant; e.g. the alanine variants were not "improved" in any way. In cases were the biological activity was improved no significant changes in stability or receptor binding preferences were reported. In effect, these data would have taught away from the intended objectives of the present invention, i.e. increasing the (thermal) stability of TRAIL or modifying the receptor binding preference of TRAIL.

Accordingly, we request that a positive IPER be issued.

Yours truly,

GOODFELLOW, Hugh Robin

Encs. Amended claims